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I also certify that the attached copy of the request for grant of a Patent (Form 1/77) bears an amendment, effected by this office, following a request by the applicant and agreed to by the Comptroller-General.

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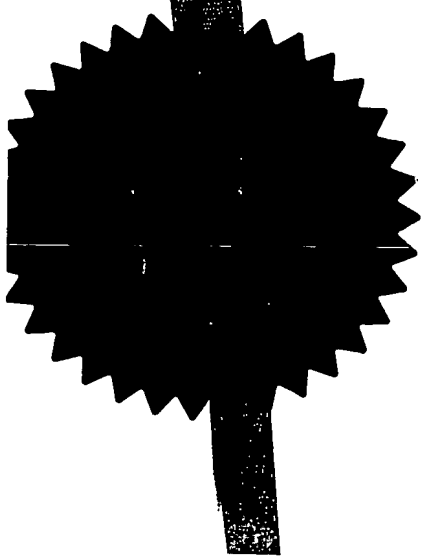

Signed Andrew Gersey

Dated 24 June 2004

Patents Form 1/77

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16JUN03 E815176-1 B11618
P01/7700 0.00-0313828.6

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

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1. Your reference

B05001

2. Patent application number
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0313828.6

16 JUN 2003

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

BRIAN DAVID OWEN OWEN-SMITH
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PO19 3EU

Patents ADP number *(if you know it)*

8653883001

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention

SALIVARY URATE FOR DIAGNOSIS OF PRE-ECLAMPSIA

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

KILBURN + STRODE
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6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*
	125001	FS1/77

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
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See note (d))

No

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	NONE
Description	~~SIX~~ FIVE
Claim(s)	ONE
Abstract	
Drawing(s)	SIX ONLY

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/~~We~~ request the grant of a ~~patent~~ on the basis of this application.

Signature B.D. Owen-Smith Date 14th June 2003

12. Name and daytime telephone number of person to contact in the United Kingdom 01243 786688

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Salivary urate for diagnosis of pre-eclampsia

DUPLICATE

Dr Brian D Owen Smith

Description

[0001] This invention relates to detection of pre-eclampsia. In particular, it relates to diagnosis of pre-eclampsia by detecting elevated levels of urate, the sodium salt of uric acid, 2,6,8, trioxypurine, in a biological fluid, saliva.

[0002] The invention is a method of using the enhanced sensitivity inherent in urate concentration in saliva, as compared with that of blood, and depends on urate as a metabolic compound with unique and predictable behaviour.

[0003] Pre-eclampsia occurs in approximately 2-4% of pregnant women and is a common cause of maternal mortality and foetal and maternal morbidity. In severe cases, early delivery may be necessary and there is therefore the risk of the child being handicapped.

[0004] Pre-eclampsia, otherwise known as 'gestational proteinuric hypertension (GPH) is a multisystem disease of pregnancy of unknown cause. The maternal syndrome is characterised by various abnormalities: increased blood pressure, oedema, proteinuria and abnormal clotting, liver and renal function. This may lead to *heart and kidney failure, intracranial haemorrhage and fits. The cause of pre-*eclampsia is believed to lie in the placenta and there is evidence for a circulating endothelial cell "toxic" factor.

[0005] Oxidative stress is believed central to development of pre-eclampsia and associated with poor placental perfusion. Raised blood urate, a marker of oxidative stress, is associated with severity of pre-eclampsia and foetal outcome (Many et al, 1996). Blood urate is also a predictor of pre-eclampsia (Chappell et al 2002).

[0006] The symptoms of pre-eclampsia are generally detectable from around 28 weeks up to full term and are not usually apparent before 24 weeks. The conventional tests, as disclosed in the prior art EP 0927355B1 are for kidney failure by measuring urea in the blood or protein in the urine, and for increased maternal *blood pressure.*

[0007] The prior art however fails in several important aspects, blood tests are time consuming, need an experienced operator, may cause physical and psychological discomfort and could lead to dangerous needle stick injuries such as infectious hepatitis. Blood samples also require specialist storage techniques to arrest decomposition.

[0008] What is needed therefore is a method for identification of those pregnant women at risk of pre-eclampsia with a biochemical test to detect oxidative stress, that is of high sensitivity, is inexpensive, non-invasive and is both simple and quick to perform routinely or in emergency and is safer, easier, quicker, and cheaper to obtain than blood.

[0009] A test for urate is ideal since it is a powerful antioxidant and marker of oxidative stress. Further, biochemical change is likely to occur before irreversible tissue damage. But blood urate concentration is a poor discriminator of pre-eclampsia as it is also increases in normal pregnancy.

[0010] However, blood urate is a product of endogenous urate production, diet, renal excretion and gastro-intestinal excretion. In the intestine, urate undergoes bacterial degradation (enteral uricolysis) (Sorensen, 1960) and is subject to diurnal variability (Owen-Smith et al, 1981, Figure 1). At night, urine excretion of uric acid is reduced whereas intestinal elimination is increased. It has been shown that blood urate concentrations also demonstrate diurnal variation (Kanabrocki et al, 2000, Figure 2). The diurnal variation is thought primarily due to sleep (Owen-Smith,1983. Figure 3). The net result of these changes is relative stability of blood urate so that with overproduction of cellular urate, increase in blood concentration is delayed.

[0011] Urate is a small molecule (Molecular weight 168) and widely diffused in extracellular fluid, including saliva. Salivary urate is not reabsorbed (as in the kidney) but is swallowed and degraded by bacteria in the intestine. Thus, rapid changes of surges of urate production are detected in saliva without significant change in blood urate concentration.

[0012] It has been demonstrated that concentration of urate in saliva is a more sensitive indicator of cellular urate metabolism than is found in blood. This was established in patients suffering from gout, before and after treatment with allopurinol (that prevents urate formation) and in normal subjects undertaking exercise or providing samples day and night (to assess diurnal variability in excretion) (Owen-Smith et al, 1998).

[0013] Further, a patient with pre-eclampsia provided both blood and salivary urate samples on the days before and after delivery (Figure 4). Both sets of samples were greatly raised as compared with normal non-pregnant women and a 28 weeks pregnancy. The salivary levels in pre-eclampsia showed good correlation with blood levels. The increase in saliva concentration from 28 weeks pregnancy to delivery was 180µmol/l.

[0014] The present invention provides in one aspect a diagnosis of pre-eclampsia which method comprises measuring urate in a biological sample for comparison with normal values.

[0015] The sample is maternal salivary urate, which has not previously been used for diagnosis of pre-eclampsia in pregnancy.

[0016] The invention may also diagnose conditions of oxidative stress from other causes and may assess activity or severity of pre-eclampsia.

[0017] The ease of collection of saliva and assay of urate fulfil the criteria of cost, safety and convenience for patient and laboratory technician.

[0018] The invention is a useful adjunct to surveillance of pregnancy and pre-eclampsia.

According to the present invention a method for testing for pre-eclampsia in pregnant human females is characterised by a method which comprises measuring urate in a biological sample.

The invention will now be described by example and with reference to the following figures.

Figure 1 Daily variation of enteral uricolysis increased at night. Graph of Disintegrations/sec/mmol $^{14}CO_2$ derived from uric acid-2-^{14}C (1.19mmol/l) injected intravenously during (A) 5 days fasting and hyperuricaemic and (B) 5 days whilst normouricaemic on a low purine diet.

Figure 2 Showing increased sensitivity and circadian variation of salivary urate concentration as compared with blood urate using serum data from Kanabrocki et al. Salivary urate correlates with serum. Serum urate (bars and left Y axis) ranges from 380-405µmol/l whilst saliva (line and right Y axis) is amplified from 125- 350µmol/l i.e. a range of 225µmol/l.

Figure 3 Showing fall in blood urate during 42 hours of sleep deprivation (while on a low purine diet) with increase during sleep and associated fall in urine uric acid excretion.

Figure 4 Blood and saliva data for non pregnant women, a normal pregnancy of 28 weeks and 3 days blood and saliva urate data from a patient with pre-eclampsia, who was delivered on day 2, showing elevations in salivary urate.

Figure 5 Showing a plastic capped Salivette® tube and cotton swab insert. Note insert vessel, which has a perforation in its base. From 0.5-1ml of saliva collects in the apex of the tube after centrifugation.

Figure 6 Showing mean normal diurnal variation of salivary urate in 4 non-pregnant females. The variation ranges from 70-190µmol/l.

SAMPLE COLLECTION

[0019] Before cleaning teeth or having a meal the woman removes the cotton wool swab (Figure 5) (1), from the sterile Salivette® tube (2). A Salivette® is a plastic disposable centrifuge tube with an insert to take a cotton swab that has a perforation at its base. This is not material to the invention but we envisage other methods of saliva sampling and testing to be developed such as dry test stick or dip stick coated with suitable reagents. The woman places the cotton wool swab under the tongue to moisten it and then gently chews upon it for about 45 seconds. This activates salivation within the mouth and the swab absorbs the saliva. The swab is then returned to the insert in the Salivette ®, which is appropriately labelled, to ensure safe storage of the sample. It is then kept cool in a normal domestic refrigerator at approximately 4°C until it is transported and processed by the laboratory.

[0020] Upon receipt at the laboratory and after laboratory and patient documentation is collected from the tube label, the Salivette® tube with the swab insert is centrifuged at 3000 revs for 5 minutes. The clear sample of mixed saliva (3) is then ready for assay. Assay may be undertaken by any method that provides a level of accuracy within +/- 5% and allow interpretation within a set of values from 30 µmol/l to 1000 µmol/l. However for completeness a method using a commercially available multi-tester, the SYNCHRON CX Systems MULTI Calibrator will now be described.

[0021] The sample is its own reagent and in conjunction with the SYNCHRON CX Systems MULT Calibrator, although intended for the quantitative determination of uric acid (urate) concentration in serum, plasma or urine, saliva is perfectly compatible with this system.

[0022] Uric acid reagent is used to measure the uric acid concentration by a timed end point method. Uric acid is oxidised by uricase to produce allantoin and hydrogen peroxide. The hydrogen peroxide reacts with 4- aminoantipyrine (4-AAP) and 3,5-dichloro-2-hydroxybenzene sulfonate (DCHBS in a reaction catalysed by peroxidase to produce a coloured product.

[0023] The Synchron CX System automatically proportions the appropriate sample and reagent volumes into a cuvette. The ratio used is one part sample to 25 parts reagent. The system monitors the change in absorbance at 920 nanometers. This change in absorbance is directly proportional to the concentration of uric acid in the sample and is used by the Synchron cx system to calculate and express the uric acid concentration.

$$\text{Uric Acid} + O_2 + H_2O \xrightarrow{\text{Uricase}} \text{Allantoin} + H_2O_2 + CO_2$$

$$H_2O_2 + \text{4-AAP} + \text{DCHBS} \xrightarrow{\text{Peroxidase}} \text{Quinoneimiine} + H_2O$$

Examples

[[0024] In the assessment of sensitivity of salivary urate as compared with blood (Figure 2) the results of 2 hourly urate concentrations over 24 hours were compared with the serum urate data of Kanabrocki et al. Salivary urate was correlated with the diurnal variation of serum urate, increased at night. The 24-hour range for serum was from 380 µmol/l to 405 µmol/l i.e. a variation of only 25 µmol/l whilst the results for saliva were amplified from 125µmol/l to 350µmol/l i.e. a range of 225µmol/l

[0025] Four non-pregnant women (Figure 6) gave salivary urate samples to demonstrate diurnal variation. There was increase in the early hours. This is must be taken into consideration in interpretation of test results and also changes in normal variation due to pregnancy. The average of the 8am to 6pm test results (76 µmol/l) show a significant lower value set than the pre-eclamptic (325 µmol/l) (fig 4).

Claims

1 A method for diagnosis of pre-eclampsia which comprises measuring urate in a biological sample.

2. A method as claimed in claim 1, wherein the sample is from maternal saliva.

3. A method as claimed in the above claims, wherein the urate is measured by means of a timed end point method.

4. A method as claimed in claim 1 or 2, wherein the urate is measured by means of a dry test method.

5. A method as claimed in any of the above claims, wherein the urate is measured by means of a dip stick method.

6. The use of urate levels in the saliva as an indicator of pre-eclampsia.

7. A diagnostic kit for performing the method of any one of the above claims, wherein the kit comprises reagents required to determine the level of the urate being measured.

8. A test equipment for performing the method in claims 1 and 2 of, wherein the test equipment comprises reagents required to determine the level of the urate in saliva being measured to determine pre-eclampsia.

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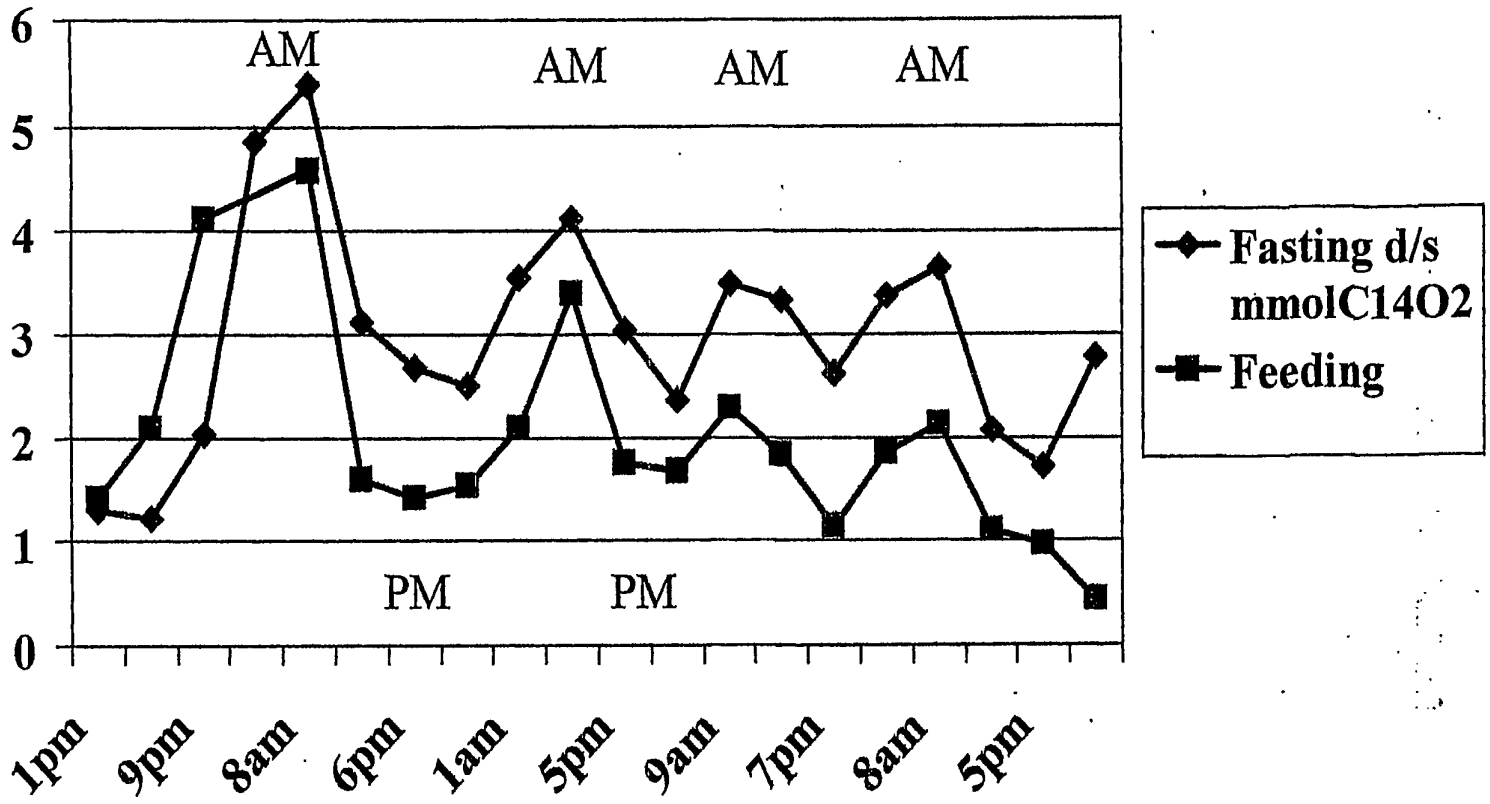


Figure 2 Comparative sensitivity of circadian variation of blood and saliva (serum data from Kanabrocki et al)

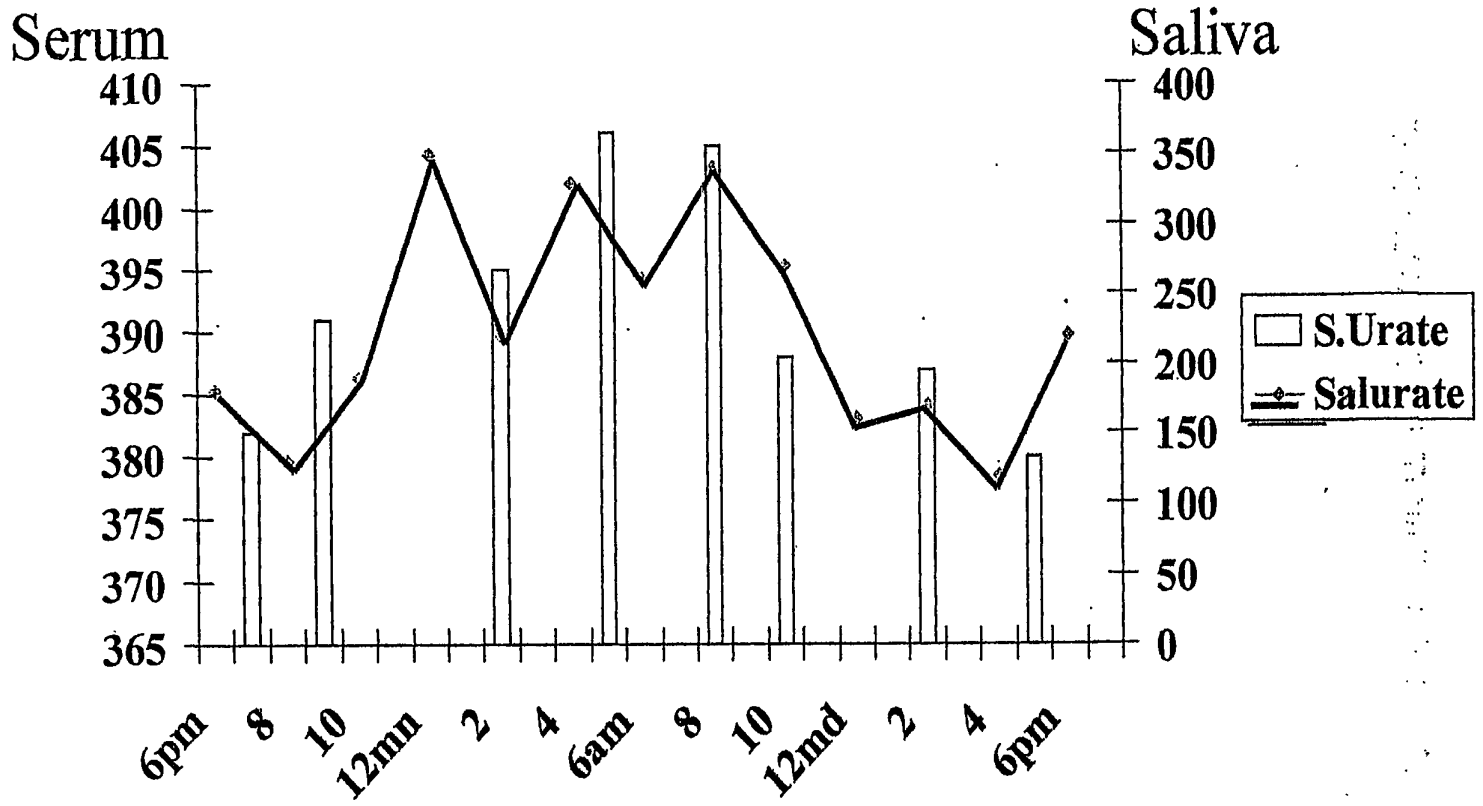


Figure 3. Effect of sleep and 42 hours sleep deprivation on blood and urine urate concentration

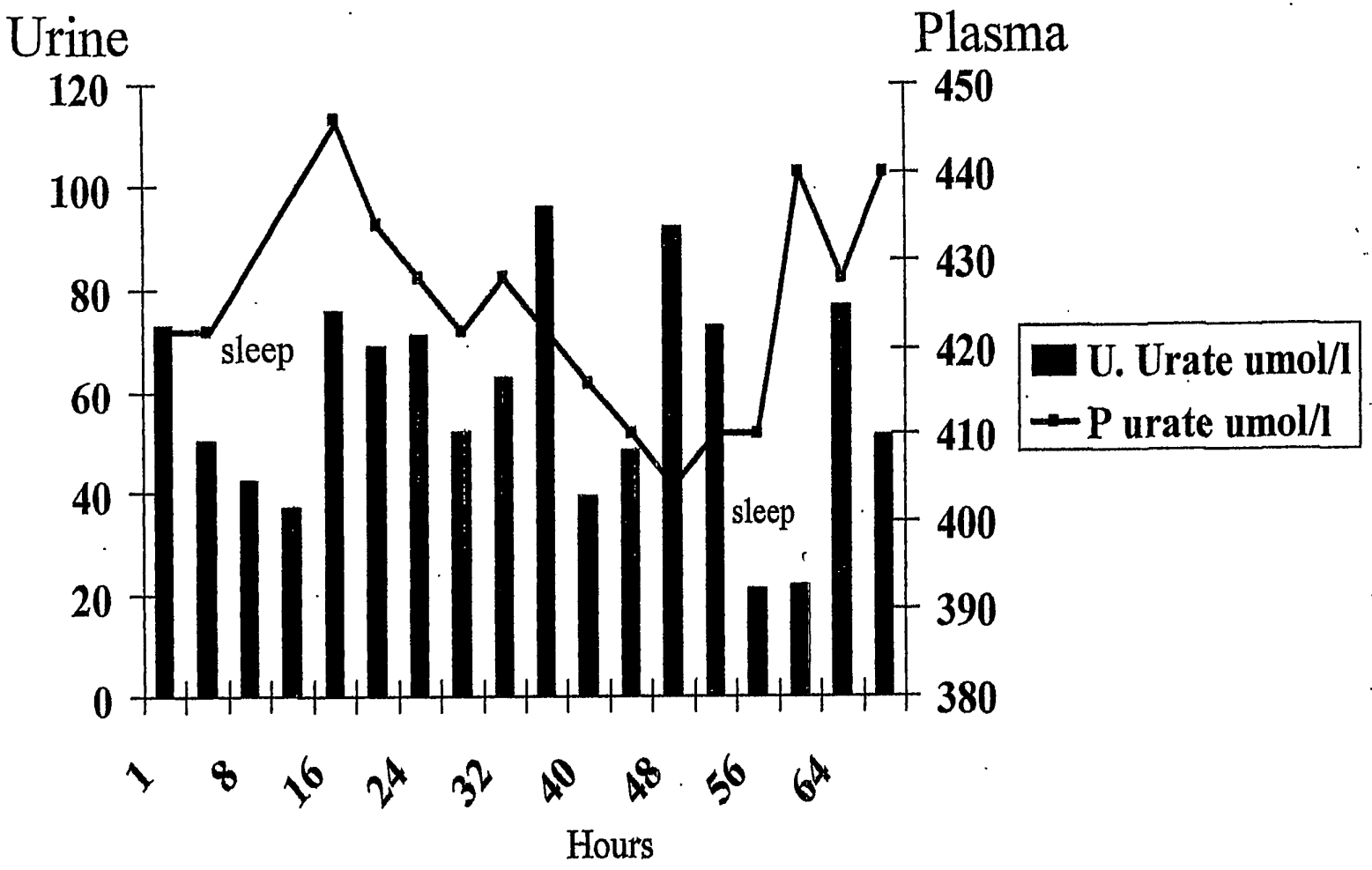
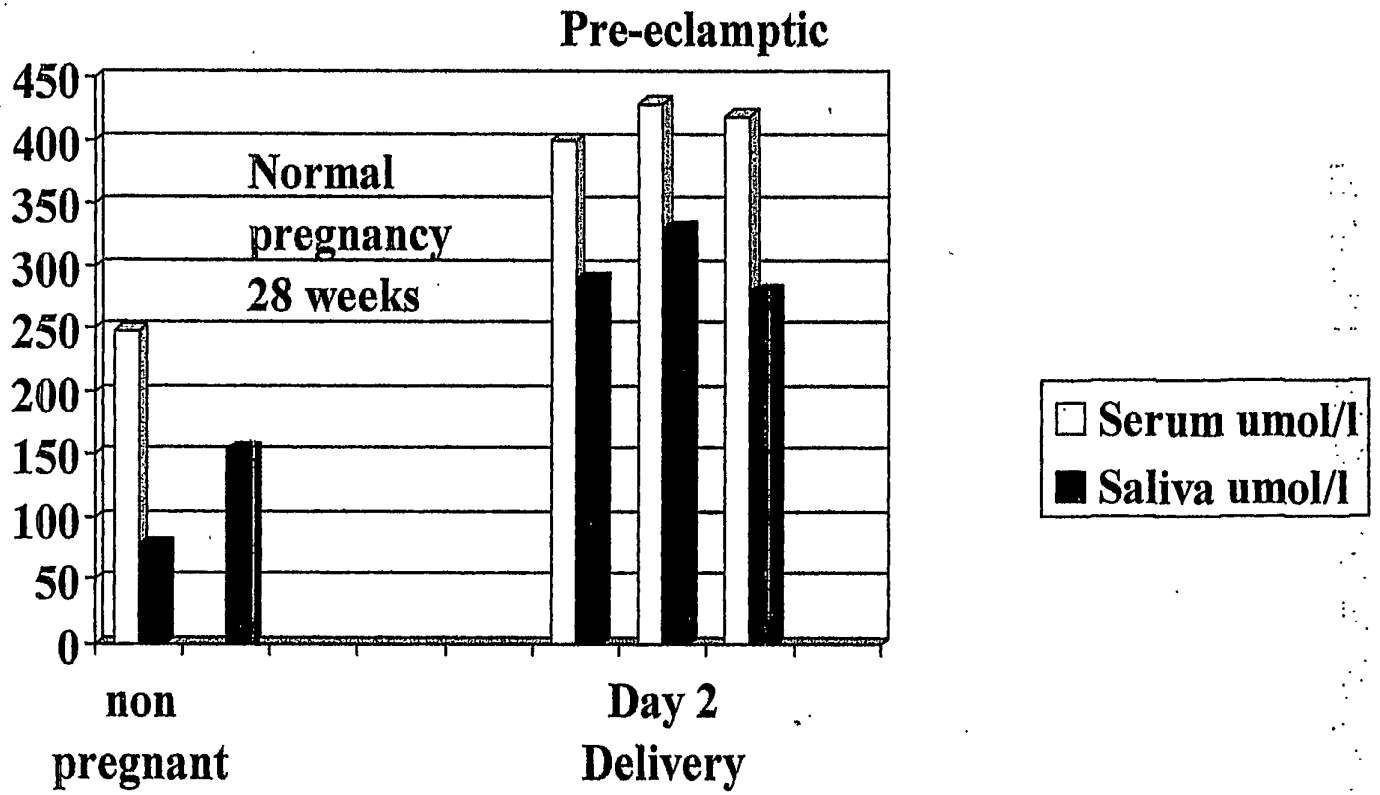


Figure 4. Showing blood and saliva data for non pregnant women, a normal pregnancy of 28 weeks and 3 days blood and saliva urate data from a patient with pre-eclampsia who was delivered on day 2, showing elevation in salivary urate.



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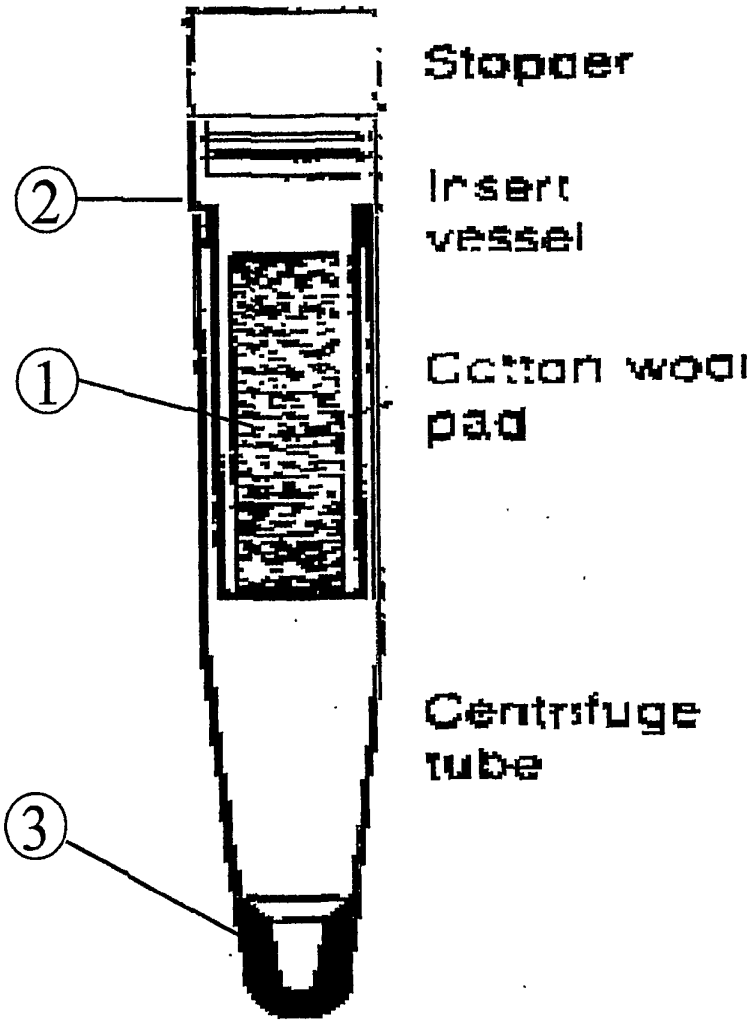

Figure 6. Mean daily variation of salivary urate in 4 non-pregnant women